Cache Creek Mercury Investigation USFWS Final Report



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I. Introduction

The Cache Creek watershed is located in the California Coastal range approximately 100 miles north of San Francisco in Lake, Colusa and Yolo Counties. Cache Creek drains eastward through arid oak woodlands and chaparral to the Cache Creek Settling basin on the valley floor and from there to the Yolo Bypass of the Sacramento River, near Woodland, California. The Cache Creek basin occupies 1150 square miles and is composed of three distinct sub-basins: the north fork of Cache Creek, the south Fork of Clear Creek and Bear Creek. The north and south forks are dammed at Indian Valley and at Clear Lake with winter storm runoff being trapped in both reservoirs for release during the summer irrigation season. Bear Creek, being undammed and not as steep has a somewhat different flow regime, making a greater contribution to downstream flows in early season storms. With the exception of the Clear Lake shoreline much of the upper basin is sparsely occupied with a xxx square mile area managed by BLM as a "primitive area".

Wildlife resources in the Cache Creek drainage include the second largest wintering population of bald eagles in California, peregrine falcons, tule elk, river otters, foothill yellow legged frog and other wildlife. A total of 154 species of birds have been observed in the watershed (BLM web site, Bird Check list for Cache Creek). Waters within the Cache Creek Watershed are typically warm and alkaline, consequently the predominant fish resources in Cache Creek are members of the minnow family (Cyprinidae) such as pikeminnow (*Ptychocheilus grandis*), Hitch (*Lavinia exilicauda*), and California Roach (*Hesperoleucus symmetricus*) as well as Sacramento Sucker (*Catostomus occidentalis*), catfish and largemouth bass.

The Cache Creek watershed has been identified as a dominant Coast Range source of mercury to the Sacramento River and the Delta (California Department of Fish and Game 1988 CVRWQCB, 1998). There are three inactive mercury mining districts in the upper Cache Creek Basin, the Clear Lake district, the Wilbur district and the Knoxville district, each of which has a number of abandoned and un-reclaimed or partially reclaimed mercury mines including one superfund site at Clear Lake. Currently there is an interagency effort to evaluate these mines within each of the sub-basins for contributions to mercury loads borne by Cache Creek.

This report represents an independent effort by the Fish and Wildlife Service to quantify mercury bioaccumulation in the Cache Creek watershed. Our evaluation of mercury concentrations in biota focused upon the downstream hydrological region of Cache Creek, the upstream portion of the North for of Cache Creek and Bear Creek above and below inputs from Sulphur Creek, but did not include sampling in Clear Lake as this area has been well characterized for mercury bioaccumulation by others (Suchanek et al, 1993 and 1995).

Since 1976, the State of California's Toxic Substances Monitoring Program has consistently reported high mercury concentrations in Cache Creek (California Department of Fish and Game 1988). Within the Cache Creek Watershed several waterbodies (Cache Creek, Clear Lake and Sulphur Creek) are listed by EPA as impaired due to mercury contamination USEPA, 1998). During the 1995 and 1997 floods in Northern California, very high mercury concentrations were measured in water samples in lower Cache Creek, the Yolo Bypass, and the Sacramento Delta (Central Valley Regional Water Quality Control Board 1998). These data indicated that large-scale, downstream movements of mercury were occurring in the Cache Creek watershed. Resources in the Sacramento-San Joaquin River Delta (Delta) and San

Francisco Bay Estuary may be threatened by mercury transport and deposition from the Cache Creek watershed. Despite documentation of elevated mercury in water, the ecological risks of mercury upon the fish and wildlife species inhabiting Cache Creek watershed itself have remained poorly characterized.

One established source of mercury is Clear Lake whose outfall forms the headwaters of the mainstem of Cache Creek. An estimated 100 metric tons of mercury were deposited into Clear Lake during nearly a century of mining at the nearby Sulphur Bank Mercury Mine, now a superfund site (Suchanek et al. 1995). Bed sediment concentrations as high as 250 parts per million (ppm) have been measured in the lake (Suchanek et al. 1995) and total mercury concentrations in water samples range from 3.6 to 104 ng/L (Gill and Bruland 1990). Davis Creek Reservoir, whose outfall also flows into Cache Creek, is a second documented source of mercury in the watershed (Gill and Bruland 1990). Invertebrates and fish in the Davis Creek reservoir contain very high concentrations of mercury (Slotton *et al.* 1995, 1997). Several abandoned mines and active springs and vents are on the same fault line as the Sulphur Bank mine and drain into Cache Creek from downstream tributaries in Colusa and Lake Counties including Sulphur Creek, a tributary to Bear Creek that drains the Wilbur Mining district and also includes contributions form geothermal springs enriched with mercury.

Analyses of mercury residues in benthic macroinvertebrates collected in the Bear Creek drainage by Slotton (1997) provided a preliminary indication that some of the smaller tributaries of Bear Creek are heavily contaminated by mercury. A substantial amount of the mercury flowing into Bear Creek can also be attributed to inputs from the Sulphur Creek watershed.

Given the wide distribution of mercury sources and the preliminary results of Slotton (1997) we

established a sampling goal of assessing mercury bioaccumulation in the lower Cache Creek watershed to assess within watershed mercury risk and evaluate relative mercury bioaccumulation risks in the North Fork Cache Creek, Sulphur Creek, Bear Creek and lower Cache Creek sub-watersheds.

II. METHODS

II.A. Sample Collection and Preparation

We collected invertebrates, fish, amphibians, and bird eggs from the Cache Creek watershed in the spring and early summer of 1997 and 1998 (Tables 1 and 2, Figure 2). In 1998, water quality measurements were taken at each site where fish were collected (Table 3). Water quality parameters were measured with a Hydro-lab® Datasonde3 and included temperature, pH, dissolved oxygen and electrical conductivity. Water quality parameters were collected in 1998 concurrent with fish collections. Sample locations, including latitude and longitude are tabulated in appendix A.

Invertebrates:

Aquatic invertebrate specimens were collected using hand held dip nets and seine nets.

Separation of biological samples from unwanted material was accomplished by using stainless steel sieves and glass (or enamel) pans pre-rinsed with deionized water then native water.

Composites were placed in Whirlpack bags for storage. Aquatic insects were collected in several watershed locations. In North Fork Cache Creek, Mill Creek, Bear Creek, Sulphur Creek, and in Cache Creek near Rumsey, Guinda, Esparto, and the Woodland Airfield, were obtained to measure mercury concentrations in composite samples. Three composites of 5 grams each of aquatic invertebrates sorted by taxa were collected. Predominantly the taxa collected were

aquatic stage larvae of caddisflies (trichopterans), dragonflies (anisopterans) and dobsonflies (megalopterans). Damsel flies (zygopterans) were collected at two locations (Bear Creek upstream of sulphur Creek and Sulphur Creek.

Fish:

Several species of fish were collected from Bear and Cache Creeks in the spring and summer of 1997 and the fall of 1998. All samples were analyzed for total mercury and a subset of samples were analyzed for methylmercury and selenium. California Roach, Pikeminnow, and Sacramento Sucker were collected in the greatest numbers across all sampling sites. Common carp, goldfish, green sunfish, brown bullhead, small mouth bass, hitch, and riffle sculpin were also collected. Fish collected in 1997 were composited by species and size range while fish collected in 1998 were analyzed individually, with a sampling goal of 10 individuals per species per site.

Fish were collected with hand nets, gill nets, seines, and electroshocking equipment..

Analyses were performed on individual fish. Samples were analyzed for total mercury and a subset were also analyzed for methylmercury and selenium. Muscle plugs were taken from a few pikeminnow for comparison with whole body results. Samples were analyzed for total mercury and a subset were analyzed for methylmercury as well.

Amphibians:

USGS personnel collected most of the amphibian species. A separate report on these collections and the analytical results has been prepared by Roger Hothem of USGS but will be

considered in the discussion section of this report. Species collected by USGS included yellow-legged frog as well as bullfrog and pacific treefrog. At each site, three composite samples of five to ten tadpoles, the most sensitive stage for most amphibians, or adult amphibians, were collected. USFWS opportunistically collected amphibians during electro fishing in the Bear Creek drainages in 1998. Target species collected by FWS were foothill yellow-legged frogs, as these native frogs are candidate species with declining populations in some portions of the state. Results of these yellow legged frog chemical analyses are presented in this report. USGS results are presented separately.

Birds:

USGS personnel monitored cliff swallow nests on a series of bridges in the Cache Creek watershed. One egg or nestling from each of 12 cliff swallow nests were collected by hand from appropriate bridges over Cache Creek and its tributaries (up to 10 sites). A separate report detailing the results of the Cliff swallow eggs analyses has been prepared by Roger Hothem of USGS Biological Resources Division in Davis, California, but results will be considered in the discussion section of this report. Three killdeer eggs were collected by FWS personnel in the Bear Creek watershed in 1997, including one egg at Wilbur Hot Springs on Sulfur creek and two eggs on Bear Creek below the confluence of Sulphur Creek. These results are presented and discussed in this report.

II.C. Transport, Analysis and Data Interpretation

All biota samples were stored on ice while in the field and kept frozen to -20 °C during storage and shipment. Fish were evaluated in the field for overt morphological and physiological impacts and stunted growth. Trace element analyses were conducted by the Trace Element Research Laboratory (TERL) at Texas A&M University. Sample spikes, blanks, and duplicates were run with samples for quality control. All standard environmental investigation's laboratory and field Quality Assurance/Quality Control (QA/QC) were followed for this study. Total Mercury

Before samples were analyzed by the cold vapor atomic absorption spectrometry (CVAAS) method, mercury is converted to the Hg2⁺ form. Mercury is digested by a modified version of EPA method 245.5 and 245.6. Tissue samples can be analyzed either freeze dried or on a wet basis. Tissue samples were homogenized in the original sample containers either after freeze drying or with a Tekmar Tissumizer and subsampled. Samples were digested with nitric acid, sulphuric acid, potassium permanganate, and potassium persulfate in polypropylene tubes in a water bath at 90-95 EC. Before analysis, hydroxylamine hydrochloride was added to reduce excess permanganate and the samples were brought to volume with distilled-deionized water.

In the CVAAS procedure, divalent mercury (Hg⁺⁺) in aqueous tissue samples was reduced to the elemental state (Hg^o) by a strong reducing agent (stannous chloride). Gaseous Hg^o enters the sweep gas and is introduced into an atomic absorption cell, where light produced by a mercury vapor lamp is absorbed by the free Hg atoms. Mercury in the sample is determined by comparing light absorption of the sample with that of external calibration standards.

Methylmercury

The procedure used to extract organo-mercury compounds in the Trace Element Research

Laboratory follows the method of Uthe et al. (JAOAC 55: 583-589, 1972), and measures the sum of all organo-mercury species extracted into the solvent. This determination is essentially equivalent to the GC method for analyzing MeHg in fish muscle tissue (where almost all of the organo-mercury is present as MeHg). In other organs, such as kidneys, much of the organic mercury may be present as a form other than MeHg, and may not be measured by methods that employ detectors that are specific for halogenated compounds. Samples are analyzed either wet or after freeze-drying. Homogenized aliquots are extracted into an organic solvent with potassium bromide and copper sulfate added to improve partitioning between phases. The organic phase is digested in combusted glass vials, using nitric and sulphuric acids and potassium permanganate, in order to convert all mercury species to ionic mercury and to remove traces of organic solvent that would otherwise impact the measurement. Analysis was based upon the cold vapor atomic absorption method, although cold vapor atomic fluorescence was used when lower detection limits were required.

Two fish samples had unusually low percentages (22% and 25%) of methylmercury when results were plotted in a scatterplot of methyl vs total mercury and a reeanlysis of these samples was requested. A problem was found in the total mercury analysis of one sample and the methylmercury analysis of the other. Samples were reground to get a more representative aliquot. An errant dilution factor may have been the case in the other sample.

Selenium

Biological tissue samples were wet digested with nitric acid and converted into acidic digest solutions for analysis by atomic spectroscopy methods. When possible, tissue was freeze dried in order to minimize loss of analytes and to facilitate subsequent sample preparation steps,

and then homogenized to a fine powder by ball-milling in plastic containers. Approximately 0.20 to 0.25 g of powdered tissue is weighed into a Teflon reaction vessel and 3 ml of HNO₃ are added. The closed reaction vessel is heated in a 130 C oven until digestion is complete.

Samples are then diluted to a final volume of 20 ml with quartz distilled water and stored in 1 oz. polyethylene bottles for analysis by instrumental techniques.

Aqueous tissue samples (sample digests) were analyzed for selenium by atomic fluorescence. In this method, analytes are introduced to the gas phase by reaction with a strong reducing agent (e.g. sodium borohydride), and free atoms are bombarded with light of element-specific wavelengths. Light that is released via atomic fluorescence is measured by a detector set at a right angle to the source. Because of the low background signal, AFS is extremely sensitive and is appropriate when other methods (e.g. GFAAS) lack the sensitivity to determine ambient concentrations. Spectral interferences are few, but the method is subject to chemical and matrix interferences that may impact the hydride generation step.

Samples for which no analyte was detected were assigned the value of one-half of the detection limit for computational purposes. Mercury and methylmercury concentrations are reported on a wet weight basis. Selenium concentrations are reported on a dry weight basis. Trace element concentrations detected in this study were evaluated by comparisons to criteria, guidelines, and other research to determine whether concentrations could be hazardous to fish and wildlife. Statistical analyses were conducted with Statistica TM '99 Edition (StatSoft 1999) and Statistica Power Analysis (Steiger, 1999). Statistical comparisons using one way ANOVA procedures were done with dry weights. Fish mercury results in wet weight are presented graphically for comparison with the wet weight toxicity thresholds.

III. Results

Water Quality

Water quality data from the fall 1998 fish collection sites is summarized in table 3. Conductivity measurements ranged from 345 to 15000 with sulphur creek having the maximum reading. Electrical conductivity in the North Fork of Cache Creek is far below levels in Cache and Bear Creeks. There is a substantial elevation in electrical conductivity in Bear Creek due to inputs from Sulphur Creek and perhaps other local tributaries. Conductivity in Cache Creek declines significantly down stream. No data from Cache Creek above the

Table 1. Water quality data from 1998 fish sampling locations.

			Water Quality	Parameters	
Date	Site	Conductivity (FS/cm)	Dissolved Oxygen (mg/L)	рН	Temperature (EC)
10/23/98	Bear Creek above Sulphur Crk	932	10.59	8.64	18.25
10/23/98	Sulphur Creek above Bear Creek	15000	7.63	8.33	16.63
10/23/98	Bear Creek below Sulphur Creek	2788	8.68	8.4	12.5
11/17/98	Bear Creek @ Highway 20	3262	11.73	8.46	11.5
11/16/98	Cache Creek @ Guinda Bridge	810	9.46	8.18	12.74
11/17/98	Cache Creek @ Capay Dam	812	9.46	8.18	12.41
11/17/98	N. Fork Cache Creek	345	11.35	8.56	12.9

confluence with Bear Creek were collected so interpretation of the impacts to conductivity downstream in Cache Creek are limited. Dissolved oxygen varied between sites, with values

ranging from 7.63 mg/L to 11.73 mg/L. The lowest value was obtained in Sulphur Creek below the Wilbur Hot Springs. The Cache and Bear Creek drainages were characterized by slightly alkaline water chemistry as pH ranged from 8.18 in the lower reaches of Cache Creek to 8.64 in Bear Creek above the Sulphur Creek confluence. Water temperature in lower Cache Creek, the north fork of Cache Creek, and lower Bear Creek are similar and ranged from 11.5 EC to 12.9 EC. Bear Creek above Sulphur Creek and Sulphur Creek were both markedly higher 18.25 EC and 16.63 EC, respectively. The elevated electrical conductivity and temperature in sulphur creek could likely be due to the influences of discharges from geothermal springs.

INVERTEBRATES

Mercury in invertebrates

Total mercury was analyzed in all samples submitted for analysis while methylmercury and selenium were analyzed in a subset of submitted samples. All samples had quantifiable concentrations of all three analytes. A summary table of concentrations is presented below (Table 4).

Total mercury dry weight means ranged from 0.0734 ppm in North Fork Cache Creek in Trichopterans to 9.35 ppm in Sulphur Creek in Zygopterans. There were no significant differences between sites for Trichopterans, Anisopterans, and Megalopterans except for Harley Gulch which was statistically different at the 0.05 level from all sites (One-way ANOVA F=4.061, p=0.0062; Neuman-Kuels post hoc comparison @ %=0.05).

Aquatic invertebrates appear to be excellent biomonitors for total mercury. The results revealed a pattern of lower total mercury concentrations (<0.5 ppm dry wt.) in North Fork Cache

Creek, Mill Creek, Bear Creek upstream of Sulphur Creek, and Cache Creek near Rumsey, Guinda, Esparto, and the Woodland Airfield, but much higher concentrations in Bear Creek downstream of Sulphur Creek (0.5 to 5.2 ppm dry wt.) and in Sulphur Creek (5.0 to 8.7 ppm dry wt.) and Harley Gulch, a non-fish bearing tributary to Cache Creek. Zygopteran larvae total mercury concentrations were 25 times higher at Sulphur Creek than in Bear Creek above the Sulphur Creek Confluence, confirming observations in fish and water than Sulphur Creek is a major source of bioavailable mercury to the watersheds below. Trichopteran mercury concentrations were greatest in the lower reaches of Bear Creek When our invertebrate results are compared with data reported in prior watershed studies (Slotton, 1997), invertebrate body burdens of mercury closely agree where taxa are the same and sites overlap.

Methylmercury in invertebrates

Methylmercury concentrations in invertebrates often did not track well with total mercury concentrations. Methylmercury as a percentage of total mercury concentrations in invertebrates averaged 66% but varied between 7% and 115% . We observed that as total mercury increased, often the percentage of methylmercury declined ($R^2 = 0.62$; p = 0, dry weight slope = -6.89).

Selenium in invertebrates

Selenium in invertebrates was only analyzed in four samples, 3 megalopteran and one damselfly larvae sample. Concentrations varied from 0.96 to 4.6 ppm with a mean of 2.3 ppm (dw). The maximum concentration found in megalopterans in Lynch Creek.

Table 2. Concentrations of total mercury, methylmercury (**bold**), and selenium (*italics*) in composite invertebrate samples. Hg and MeHg are reported in ppm wet weight and Se in ppm dry weight.

<u>Site</u>	Trichoptera	<u>Plecoptera</u>	Zygoptera	<u>Anisoptera</u>	<u>Megaloptera</u>
Bear Creek Sample Sites					
Mill Creek @ Brim Road	0.0356			0.0352	
	0.0326			0.0324	
Bear Crk above Sulphur Crk			0.0692		
Bear Crk below Sulphur Crk	0.103			0.077	0.495
	0.062			0.0578	
Sulphur Crk above Wilbur	0.348		1.515	1.349	
Springs Rd	0.0345			0.180	
				2.53	
Bear Crk @ Hamilton Canyon	0.0261			0.0363	
	0.0166			0.0303	
Lynch Canyon					0.032
					0.027
					4.68
Bear Crk @ Hwy 20	0.429			0.433	0.245
	0.176			0.259	0.168
					1.04
Bear Crk @ 15-37	0.534			0.235	0.204
	0.255			0.159	0.151
Thompson Canyon					0.014
					0.0049
<u>Un-named Trib 1 to Bear Crk</u>					0.027
					0.022

<u>Un-named Trib 2 to Bear Crk</u>		0.012	 	0.0256
				0.0202
Cache Creek Sample Sites				
<u>Cache Crk @ 22-19</u>	0.028		 0.013	0.030
	0.0161		0.0155	0.0236
				0.957
Cache Crk @ Esparto	0.0302		 0.035	
	0.027		0.0386	
Cache Crk @ 94B	0.0193		 0.0091	
	0.0131		0.0100	
Guinda Bridge	0.017		 	
	0.0163			
Harley Gulch			 	1.431
				0.101
NF Cache @ Hwy 20	0.0057		 0.015	
	0.0045		0.0124	

FISH

Mercury in fish

In April and August 1997, composite samples of California Roach (an herbivore and insectivore between 6 and 12 cm) were collected in Bear Creek upstream and downstream of Sulphur Creek. In both seasons, whole-body mercury concentrations were much higher in fish collected downstream of Sulphur Creek (3 times greater than upstream concentrations in April and 4 times greater in August). In both upstream and downstream sites, samples collected in August contained much higher mercury concentrations than those collected in April (upstream concentrations in August were 0.4 ppm (wet weight) and in April were 0.1 ppm; downstream concentrations in August were 1.7 ppm and in April were 0.3 ppm.). Because of these

significant accumulations through the 1997 summer season our 1998 fish collections were scheduled for the fall. The fall sampling strategy maximized both the logistics of fish collection via enhanced stream access at low flows and the seasonal effects of maximal mercury bioaccumulation.

In the fall of 1998 individual Roach were collected in numbers sufficient to permit statistical comparisons between four sites, Bear Creek above Sulphur Creek, Bear Creek below sulphur creek, Bear Creek at Highway 20 and Cache Creek above Capay Dam. Mean wet weight concentrations of mercury in roach at the four sites were 0.36, 0.42, 0.87 and 0.24 mg/kg, respectively. A one way analysis of Variance indicated a highly statistical difference in dry weight mercury concentrations. (F = 19.43 and p = 0). A post-hoc comparison using Tukey's honest significant difference test indicated roach mercury concentrations in Bear Creek at Highway 20 were quite significantly elevated above the other three sites (p < .0002).

Sacramento suckers were only collected in 1998 but were found in sufficient numbers to permit statistical treatment at six locations. Application of ANOVA test to means for the six locations indicated mean concentrations between sites were different (F = 63, p = 0). Mean dry weight concentrations of mercury in suckers ranged from 0.20 to 4.54 mg/kg with the North Fork of Cache Creek having the lowest mean and Bear Creek at Highway 20 having the highest mean mercury concentration. Suckers at Bear Creek downstream differed from all other locations as did suckers at Bear Creek at Highway 20. Average wet weight concentrations in Suckers were below 0.1 ppm at Guinda and the North Fork Cache Creek. Suckers at Bear Creek a mile downstream of Sulphur creek had mean wet weight mercury above 0.3 ppm and further downstream at highway 20 mean concentrations exceeded 1.0 ppm on a wet weight basis.

Pikeminnows were collected in 1998 at five locations. These sites, in decreasing order of mercury concentrations were Bear Creek at highway 20 (n= 1) Bear Creek upstream of Sulphur Creek, Capay Dam, Guinda Bridge, and the North Fork of Cache Creek. Mean wet weight mercury concentrations were 1.65, 0.58, 0.28, 0.16 and 0.09 ppm respectively. A one way analysis of variance indicated significant differences between these groups (F = 9.49; p = .000026). Post hoc comparisons revealed all groups differed from one another except the Bear Creek sites, although only one fish was collected at the Bear Creek highway 20 site. The magnitude of the difference between this individual and upstream pikeminnows and results of other biota sampling suggest that almost certainly more samples would have revealed differences between pikeminnows at highway 20 and upper Bear Creek if we could have found and collected them. Wet weight concentrations in pikeminnows exceeded 1.5 ppm at Bear 20 and 0.5 ppm at the Bear Creek site upstream of sulphur creek and were near 0.3 ppm at Capay dam. Guinda and North Fork Cache Creek pikeminnows had wet weight mercury concentrations of 0.16 and 0.10 ppm, respectively.

For all three species of fish, mercury concentrations varied with length of the fish, but location not length was clearly the most important factor determining fish mercury concentrations. Length differences between sites were only found for Sacramento Suckers with fish and at Guinda being the largest. Suckers at Guinda however had mean dry weight mercury concentrations an order magnitude lower than the Bear Creek highway 20 site. Mercury concentrations, as they varied by fish length, species and location are depicted in two color graphics in Appendix D.

Within site comparisons between species indicated Pikeminnow - the largest and most

piscivorous fish - was consistently the fish species with highest mercury concentrations. At the more contaminated sites in Bear Creek , Suckers were the second most contaminated but Roach were more contaminated than Suckers at Bear Creek upstream of Sulphur creek and at Capay dam, despite the fact that suckers here were much larger on average and probably older than the Roach. Capay however did have statistically larger roach than at any other site (p < .02), perhaps slightly boosting mercury concentrations here in Roach.

Fish Muscle plugs vs. whole body result

In comparisons of dry weight total mercury concentrations in four muscle plugs with whole bodies of fish we consistently found mercury concentrations in the muscle plugs to be higher in total mercury than whole bodies but results of a dependent sample t-test indicated the probability of this occurring by chance was p=0.111. Ordinarily this would call for an acceptance of the null hypothesis, that is muscle plugs and whole bodies do not differ in mean total mercury concentrations. A power analysis however, indicated an 83% chance of a type II error in accepting the null hypothesis at this sample size. If we were to test this hypothesis in the future a sample size of at least 19 would be needed to confidently ($\beta=0.2$) accept the null hypothesis of no difference between muscle plugs and whole body mercury concentrations. We therefore conclude for now that it is more likely than not that muscle plugs reflect a higher concentration of total mercury than do whole bodies.

Methylmercury was analyzed in two the individuals with muscle plug data. The muscle plugs had a higher percentage found as methylmercury (just over 100%) vs whole body samples from the same individuals (70%). Correlations between whole body measurements and muscle plug results for total mercury were also not statistically significant ($R^2 = .25$, p = 0.49).

A summary table of mean concentrations in whole individual fish from 1998 is presented below (Table 5). Results of all residue analysis for all fish species is presented in appendix B.

Methylmercury in fish

Methylmercury was analyzed in 21 individual fish for comparison with total mercury values. Methylmercury concentrations were on average 90% of the total mercury value with a 95% confidence interval of 82.6 to 97.4%. Methylmercury was highly correlated with total mercury among all fish ($R^2 = .94$, p = 0). The slope of the curve was 0.969. The one to one ratio line was within the 95% confidence interval of the plotted regression line for methylmercury as a function of totalmercury. We had thought we might find smaller Size or species of fish did not seem to be related in any fashion to methylmercury as a percentage of the total mercury value.

Table 3. Mean Mercury (dry weight) concentrations in fish from 1998 in the Cache Creek Watershed. Number of individual fish in the sample size are given in parenthesis.

	Pike Minnow	Sucker	Roach
Bear 20	7.59 ^A (1)	4.54 ^A (6)	2.80 ^A (10)
Bear Down		1.50 ^B (8)	1.24 ^B (9)
Bear Up	2.14 ^{AB} (10)	0.55 ^C (11)	1.09 ^B (10)
Capay	1.15 ^{BC} 10	0.68 ^c (11)	0.9 ^B (10)

Guinda	0.59 [°] (10)	0.37 ^c (12)	
North Fork	0.37 ^c	0.20 ^C	0.1 ^c
	(9)	(10)	(1)

Values with the same superscript letters depict statistically similar mean concentrations as assessed with One way ANOVA, and Tukey's Honest Significant Difference test for unequal sample sizes.

Selenium in fish

Selenium was assessed in Pikeminnows at three locations, Guinda, Capay Dam and North Fork of Cache Creek. Dry weight selenium concentrations at these three sites were 1.9, 1.4 and 3.2 ppm. The North Fork of Cache Creek had statistically higher concentrations of selenium than the other two sites. (P < .05). Selenium was assessed in Roach at three locations: Bear 20, Capay and Sulphur Creek. Mean Selenium concentrations, 2.15, 1.4 and 4.0 at these sites respectively were statistically different at Sulphur creek from the other two sites (p < .02 and P< .01). In suckers, whole body selenium concentrations were slightly, but statistically higher in the upper portions of the watershed at the North Fork and upstream Bear Creek site.

Generally, fish concentrations in excess of 4 ppm dry weight are regarded as the whole body threshold concentration for adverse effects. With the exception of roach at Sulphur Creek none of the fish sampled in this study were at or above that value.

Birds

Three random killdeer eggs from the Bear Creek region were collected from each of three nests in 1997 for mercury determinations. Mercury concentrations in wet weight were 0.26,

from a nest near the Jones "Fountain of Life" on Sulphur Creek and 0.10 and 0.90 ppm from a pair of nests on Bear Creek located about 1 mile downstream of the Sulphur Creek confluence. Brine flies in the vicinity of the Sulphur creek nest were abundant and composed the likely prey of the killdeer nesting there. Cliff swallow eggs collected by Hothem in 1997 and 1998 did not exceed 0.2 ppm ww, but mercury concentrations in eggs were correlated with observations in resident amphibians from the same areas (Hothem, 1999.)

Amphibians

Six adult yellow legged frogs from Bear Creek, three from upstream of sulphur Creek and three from downstream of sulphur creek had wet weight concentrations ranging from 0.075 to .538 mg/kg. The maximum concentration was observed in Bear Creek at highway 20. Mean concentrations in upstream frogs were 0.11 and 0.31 in downstream frogs. Sample size was not sufficient to allow adequate statistical comparisons to reject the null hypothesis with

IV. Discussion

PATTERNS OF MERCURY BIOACCUMULATION

A 1998 study by the Regional Water Board of mercury loadings in the Cache Creek Basin indicated the majority of mercury loads were carried in winter storms with major contributions from a source below the confluence of the North Fork and south fork of Cache Creek but above Bear Creek (CVRWQCB, 1998). Harley Gulch, which drains the unreclaimed Turkey Run Mine, was thought to be dominant source of mercury in this portion of the basin. During large

storms the mercury loads from the upper Cache Creek basin were between 25 Kg/day and 64 Kg/day, while during the summer irrigation season mercury loads are between 0.1 to 1 kg/day (CVRWQCB, 1998).

Loads from Bear Creek are largely influence by Sulphur creek but other undocumented sources are suspected. (Sulphur Creek for example on 2 February 1998 had mercury concentrations of 11,421 ng/L - 3 orders of magnitude EPA's aquatic life criteria). Sulfur Creek drains the Wilbur mining district which includes the Elgin Mine, the Wide Awake Mine, Abbot Mine and Empire Mine. Bear Creek loads are typically a smaller part of the Cache Creek load but Bear Creek at least in some years may contribute more of the mercury load in early season storms (CVRWQCB, 1998) when the other two tributaries with reservoirs are in storage mode. Concentrations of total mercury in water in all sub-basins while variable were generally high and almost always above 12 ng/L, with sometimes extremely high concentrations of several thousand ng/L found during storm events.

We found by sampling individual fish in 1998 that we were able to statistically distinguish Bear Creek from other sub-basins. Whole body mercury concentrations in pikeminnows, suckers and roach were all significantly elevated in Bear Creek and the concentration of mercury in each of these species increased as sampling progressed downstream from above the confluence with sulphur creek to our furthest downstream sampling station on Bear Creek, just above highway 20. Where sampling proceeded downstream below highway 20 in the Bear Creek drainage mercury concentrations continued to rise. The pattern in invertebrates was the same as in fish. Mercury concentrations in tricopteran and anisopteran larave increased in Bear Creek below Sulphur Creek. Yellow Legged frog comparisons in Bear

Creek above and below the Sulphur Creek confluence also hinted at a downstream increase although a slightly larger sample size would be needed for statistical confirmation.

The pattern of downstream increases of mercury in biota in Bear Creek is the opposite of that observed in Cache Creek, where mercury bioaccumulation appears to attenuate in the downstream reaches. Some possible explanations may lie in differences in hydrological patterns of flow in these two sub-basins and may also be suggested by the sharp rise in mercury in Bear Creek Roach we observed in the spring and summer of 1997. Bear Creek is a gentler drainage, without an upstream reservoir to supply summer flows. The South fork of Cache Creek is fed by Clear Lake releases for downstream irrigation as is the North Fork of Cache Creek from Indian Valley Reservoir. Thus summer flows in Bear Creek are typically quite low - usually less than 2 CFS while flows in the other tributaries are in the high 100s of CFS. As a consequence, Bear Creek's quite shallows and pools of the summertime may provide an ideal place to methylate mercury laden sediments deposited from winter flows.

TOXICOLOGICAL SIGNIFICANCE OF FINDINGS

Selenium concentrations in whole body fish in excess of 4 ppm are considered above the threshold for adverse effects to fish. Only one fish sample, a single roach individual found in sulphur creek was found at this concentration. Sulphur Creek, as the discerning reader may gather from the name, was not prime fish habitat. Warm, alkaline, mercuriferous and dominated by geothermal springs and unreclaimed mine runoff, Sulphur Creek is today of aquatic significance mostly for its ability to adversely influence downstream resources. Dietary concentrations in excess of 3 ppm (dw) may lead to an accumulation of selenium by predators. Only two samples, the above mentioned roach at Sulphur Creek and a composite of

megalopteran larvae at Lynch Canyon a downstream tributary to Bear Creek near highway 20. For the most part selenium concentrations in the Cache Creek basin were selenium normal and not toxicologically significant.

In contrast to selenium, mercury concentrations in biota were elevated to concentrations of ecotoxicological significance at many locations, particularly within Bear Creek. To assess the ecotoxicogical significance of mercury we use as our target the Common Merganser (Mergus merganser). The Common Merganser is a common year round resident of the Cache Creek and Bear Creek watersheds that breeds in the riparian zone and forages upon aquatic life, primarily fish. Fish taken by the merganser range from 6 to 36 cm, most commonly 10 to 30 cm, with larger fish preferred. Appendix D contains the details of the risk assessments supporting calculation and assumptions. The threshold concentration in fish for adverse effects to reproduction of the merganser was calculated in that assessment to be 0.27 mg/kg methylmercury (wet weight). If one assumes fish mercury is only 90% methylmercury as was the estimate in this study - independent of fish size, then the total concentration in fish estimated to be associated with a reproductive problem for mergansers would be 0.3 ppm total mercury. The no effect concentration for birds is not known. Thus to calculate a safe concentration for mercury in fish for birds, EPA, in its mercury report to congress, divided the Lowest Observed Adverse Effect Concentration by a factor of three. Applying that logic we will assume here that concentrations less than 0.1 mg/kg total mercury on a wet weight basis are safe for the Merganser. The mean of 26 location and fish species averages in our sampling of the Cache Creek Basin was 0.31 mg/kg. This sampling however was a biased, not random assessment of the entire basin. When we apply these interpretive concentrations to examine specific locations

it is apparent that Bear Creek had few fish that were safe for mergansers to eat, while the five species assessed at the North Fork of Cache Creek in 1998 were all safe. Fish mercury at Guinda and Capay were solidly in the interpretive limbo between a safe concentration estimate and the toxic threshold. Unfortunately due to poor accessibility, we did not assess fish in the one other reach of Cache Creek which might also have elevated mercury. That is the reach between the confluence of the North and South Forks of Cache Creek and the confluence of Cache Creek and Bear Creek.

CONCLUSIONS:

While water monitoring by the Regional Water Quality Control Board has indicated Cache Creek tributaries within the North Fork and South Fork of Cache Creek sub-basins are responsible for the extraordinary exports of mercury loads to the Cache Creek Settling Basin and the Yolo bypass during the winter, Bear Creek appears to experience the greatest mercury bioaccumulation hazard. Bioaccumulation is enhanced in the Bear Creek sub-basin during the summer months and increases with distance downstream from Sulphur Creek. This may imply additional sources to Bear Creek besides Sulphur Creek, or enhanced bioavailability of mercury in downstream waters, or both. Source characterization and patterns of methylmercury formation in the Cache Creek watershed particularly within the instream environment of Bear Creek during the summer season are deserving of further study to assess the potential effectiveness of upstream and or downstream sediment source control in the Wilbur Mining District. A Total Maximum Daily Load strategy that seeks to reduce biological hazards of mercury within the Cache Creek basin should focus primarily upon the Bear Creek sub-basin.

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Appendix ACache Creek Report Sample Collection Locations

1997 Sampling Sites and Specimens Collected

Site	Latitude/ Longitude	Collection Date	Matrix Collected
Bear Crk @ Hamilton Canyon	39EN 03' 24" 122EW 24' 41"	4/22/97; 5/22/97; 8/11/97	Invertebrates, Fish, Avian Eggs
Mill Creek @ Brim Road	39EN 09' 48" 122EW 26' 49"	5/22/97	Invertebrates
NF Cache @ Hwy 20	38EN 59' 21" 122EW 32' 24"	4/25/97	Invertebrates
Sulphur Crk above Wilbur Springs	39EN 02' 00" 122EW 25' 38"	4/25/97	Invertebrates
Bear Crk below Sulphur Crk	39EN 02' 20" 122EW 24' 31"	4/11/97; 5/22/97; 8/11/97	Invertebrates, Fish
Bear Crk @ Hwy 20	39EN 00' 42" 122EW 21' 24"	5/22/97	Invertebrates
Bear Crk @ Bridge 15-37	38EN 58' 19" 122EW 20' 28"	5/22/97	Invertebrates
Cache Crk @ Bridge 22-19	38EN 54' 36" 122EW 16' 42"	5/30/97	Invertebrates
Cache Crk @ Guinda	38EN 49' 43" 122EW 10' 57"	5/30/97; 6/3/97	Invertebrates, Fish
Cache Crk @ Esparto	38EN 42' 47" 122EW 00' 48"	7/7/97	Invertebrates
Cache Crk @ 94B	38EN 41' 19" 121EW 51' 52"	6/3/97	Invertebrates
Capay Dam	38EN 42' 47" 122EW 5' 00"	7/14/97	Fish
Wilbur Hot Springs			Avian Egg

Table 2. 1998 Sampling Sites and Specimens Collected

Site	Latitude/ Longitude	Collection Date	Matrix Collected
Bear Crk below Sulphur Crk	39EN 02' 17" 122EW 24' 18"	6/2/98, 10/23/98	Inverterbates, Fish, Amphibians, Killdeer eggs
Bear Crk above Sulphur Crk	39EN 03' 07" 122EW 24' 29"	10/23/98	Fish, Amphibians
Bear Crk @ Hwy 20	39EN 00' 42" 122EW 21' 24"	6/5/98	Invertebrates
Bear Crk @ 15-37	38EN 58' 19" 122EW 20' 28"	6/5/98	Invertebrates
Bear Crk below Sulphur Springs Crk	39EN 02' 17" 122EW 24' 29"	6/2/98	Invertebrates
Cache Crk @ 22-19	38EN 54' 36" 122EW 16' 42"	4/29/98	Invertebrates
Harley Gulch	39EN 00' 43" 122EW 26' 00"	4/29/98	Invertebrates
Lynch Canyon	39EN 00' 54" 122EW 23' 13"	5/18/98	Invertebrates
Sulphur Crk above Wilbur Springs Rd	39EN 02' 16" 122EW 24' 37"	5/8/98	Invertebrates, Fish Killdeer egg
Thompson Canyon	38EN 58' 33" 122EW 20' 46"	4/29/98	Invertebrates
Un-named Trib 1 to Bear Crk	39EN 00' 57" 122EW 23' 32"	5/18/98	Invertebrates
Un-named Trib 2 to Bear Crk	39EN 00' 57" 122EW 22' 48"	5/8/98	Invertebrates, Amphibians
Bear Crk above hwy 20	39EN 00' 56" 122EW 22' 47"	11/16/98	Fish
Capay Dam	38EN 47' 11" 122EW 05' 01"	11/17/98	Fish
Guinda Bridge	38EN 49' 42" 122EW 10' 50"	11/17/98	Fish
NF Cache @ Hwy 20	39EN 59' 21" 122EW 32' 24"	11/17/98	Fish

Appendix B

1998 Fish Mercury Data

Summary Table of Means of Wet Weight Mercury Concentrations

(cache98 fish.sta)

N=164 (No missing data in dep. var. list)

0 = no fish collected of this species at this location.

	Mean (ww)	N
Bear Crk Roach	.520126	9
Bear Crk Sucker	.350942	8
Bear Crk Pikeminn	0	
Bear Crk Carp	0	
Bear Crk Goldfish	0	
Bear Crk Sunfish	0	
Bear Crk Bullhead	0	
Bear Crk Sm Bass	0	
Bear Crk Hitch	0	
Bear Crk Sculpin	0	
Bear Cr1 Roach	.363195	10
Bear Cr1 Sucker	.125248	11
Bear Cr1 Pikeminn	.612004	12
Bear Cr1 Carp	0	
Bear Cr1 Goldfish	0	
Bear Cr1 Sunfish	0	
Bear Cr1 Bullhead	0	
Bear Cr1 Sm Bass	0	
Bear Cr1 Hitch	0	
Bear Cr1 Sculpin	0	
SULPHUR Roach	.336330	1
SULPHUR Sucker	0	
SULPHUR Pikeminn	. 0	
SULPHUR Carp	0	
SULPHUR Goldfish	0	
SULPHUR Sunfish	0	
SULPHUR Bullhead	0	
SULPHUR Sm Bass	0	
SULPHUR Hitch	0	
SULPHUR Sculpin	0	
Bear @20 Roach	.871553	10

1.654620	1
0	
0	
0	
0	
0	
0	
0	
.238324	10
.185319	12
.279020	10
.107219	3
.151256	1
.179667	3
0	
0	
0	
0	
.289654	1
.099861	12
.163561	10
.150920	1
0	
0	
.185232	1
.150287	2
0	
0	
.100786	1
.054147	10
.097629	9
0	
0	
0	
0	
0	
7 5	
.069855	<u>5</u>
.312742	164
	0 0 0 0 0 .238324 .185319 .279020 .107219 .151256 .179667 0 0 .289654 .099861 .163561 .150920 0 .185232 .150287 0 0 .100786 .054147 .097629 0 0 0 7 5 .069855

Appendix C

Mean Mercury (ww), methylmercury (ww) and selenium (dw) in three fish species by location.

Summary of means for total mercury, methylmercury (**bold**), and selenium (*italics*) in whole body fish samples. Hg and MeHg are reported in ppm wet weight and Se in ppm dry weight. * items are composite samples collected in 1997. Sample sizes are in parentheses.

Site	<u>Pikeminnow</u>	Roach	<u>Sucker</u>
Bear Crk above Sulphur Crk	0.577 (10) 0.451 (1)	0.363 (10) 0.333 (2)	0.125 (11) 0.082 (1) <i>1.51</i> (1)
Bear Crk @ Hamilton Canyon	0.556 (7)*	0.222 (2)*	
Bear Crk below Sulphur Crk	0.337 (3)*	0.418 (9) 1.01 (2)* 0.369 (3)	0.351 (8)
Bear Crk @ Hwy 20	1.655 (1)	0.872 (10) 0.99 (1) 2.147 (3)	1.047 (6)
Sulphur Crk above Wilbur Springs Rd		0.336 (1) 4.0 (1)	
<u>Capay Dam</u>	0.279 (10) 0.249 (3) 1.43 (5) 0.497 (6)* 1.99 (6) *	0.238 (10) 0.252 (2) <i>1.4</i> (2)	0.169 (11) 0.093 (2) 0.848 (3) 0.236 (2) plug 0.236 (1) plug 0.065 (2) plug
Guinda Bridge	0.164 (10) 0.142 (1) 1.937 (3) 0.299 (5)* 1.97 (5)*	0.290 (1)	0.10 (12) 0.756 (3)
NF Cache @ Hwy 20	0.098 (9) 0.058 (1) 3.175 (2)	0.101 (1)	0.054 (10) 1.07 (1)

APPENDIX D

Risk Assessment Calculations to establish safe and threshold adverse effect concentrations for Mercury in the Common Merganser

The following equations were used to estimate a safe mean concentration of mercury in fish eaten by common mergansers.

$$SD = TD \div 3$$

$$SD \times BW_m \div F_a = SC$$

TD = toxic dose of mercury to mallard

SD = estimated safe dose for merganser

 $BW_m = body$ weight of merganser

F_a = calculated daily consumption by merganser

SC = Safe fish concentration of mercury

Using an average body weight of 1232 g for *Mergus merganser* (common merganser) (CRC handbook of avian body masses) the food consumption per day on a grams dry weight basis may be calculated using allometric equations found in Nagy (1987):

$$g/d=.495(Bw)^{0.704}$$
. This results in $F_a=0.074$ kg food/day (dry weight)

I chose the equation for seabirds as likely best fit for common mergansers. Body weight data for female common mergansers was taken from the CRC handbook of avian body masses.

$$TD = 0.064 \text{mg/kg (bw) perday}$$

This value is from a three generation study feeding study in mallards with methlylmercury dicyandiamide (Heinz, 1979). The lowest dose resulted in adverse effects on reproduction and behavior and therefore this concentration represents a LOAEC not a NOAEC.

Since we want to know the safe concentration not the toxic concentration I divided the toxic dose by a safety factor of three to determine a "safe dose". This is consistent with the uncertainty factor applied in EPA's Mercury Study Report to Congress for all avian species where NOAECs are just not available. This results in a predicted safe dose of 0.021 mg/Kg bw per day.

$$SD = 0.021 \text{ mg/Kg bw per day.}$$

If the safe dose is multiplied by Merganser body weight the safe daily mass of mercury consumed by Mergansers is obtained (0.021 mg Hg/Kg bw per day x 1.232 kg bw = 0.026 mg Hg per day). To obtain the safe mean concentration in fish divide this mass by the daily consumption rate (0.026 mg Hg/day ÷ 0.074 kg fish/day = 0.35 mg Hg/kg fish). As the consumption rate estimated kg of dry mass in the diet (Nagy, 1987) this is a dry weight estimate. To convert to wet weight assume 75.5% moisture in fish and divide by 4.08. The result is an estimate of 0.09 ppm www as the safe concentration in fish for mergansers.

$$SD = TD \div 3$$

SD
$$\times BW_m \div F_a = SC_{dw} = 0.35 \text{ mg Hg/kg}$$

$$SC_{dw} \div 4.08 = SC_{ww}$$

TD = toxic dose of mercury to mallard

SD = estimated safe dose for merganser

 $BW_m = body$ weight of merganser

 $F_{a=}$ calculated daily consumption by merganser

SC = Safe fish concentration of mercury

 SC_{dw} = Safe fish concentration of mercury in dry weight

 SC_{ww} = Safe fish concentration of mercury in wet weight

I chose the merganser rather than the bald eagle because mergansers are likely breeding in the Cache creek drainage. The toxic endpoint for the toxic dose is reproduction.

Assumptions: only methylmercury occurs in fish and methylmercury intake from inhalation, dermal absorption, and drinking water is negligible, sensitivity of field species (western grebes and common merganser) is similar to the lab species (mallard).

To apply the above method to western grebes use 1.477 kg as the mean body weight of a western grebe. The result is essentially the same estimate for safe mercury concentration in fish: 0.09 ppm Hg in fish on a wet weight basis.

Figure 1. Sampling Locations Map of the Cache Creek Watershed. .

Figure 2.

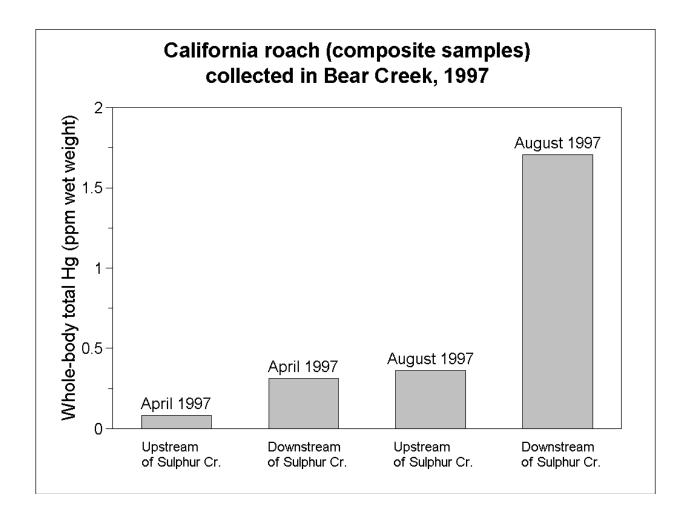
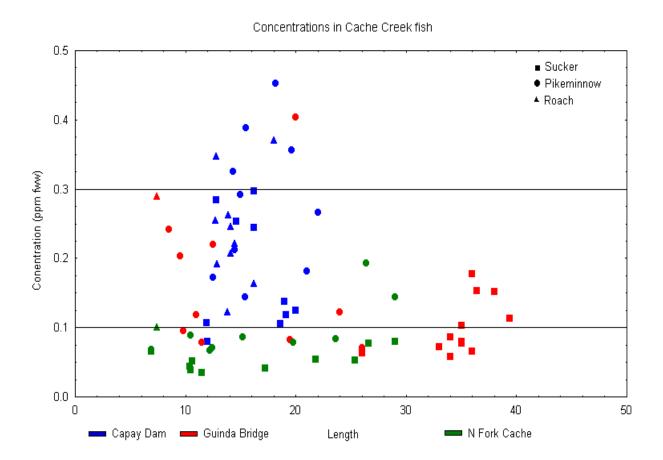
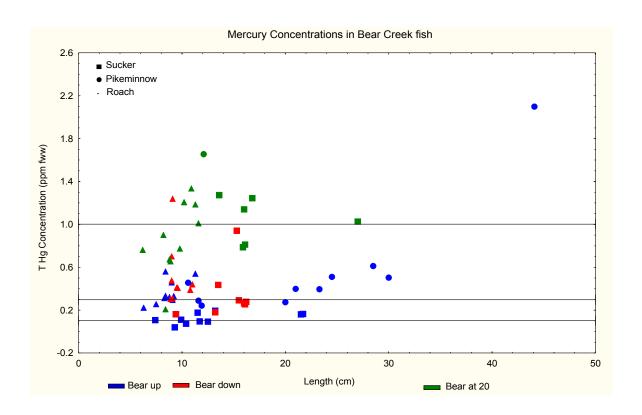


Figure 3

Figure 4.





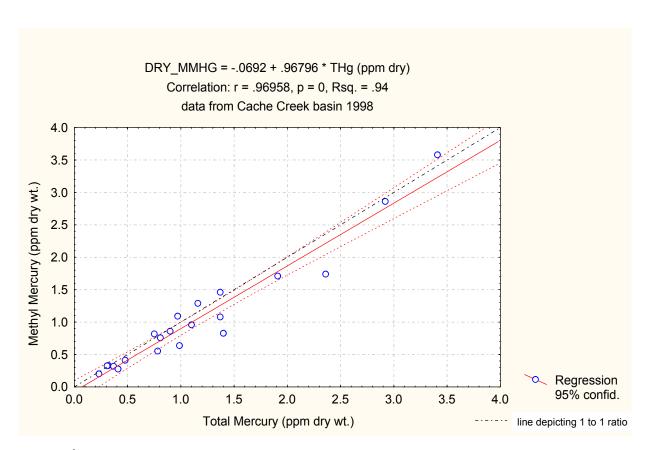


Figure 5.

Figure 6.

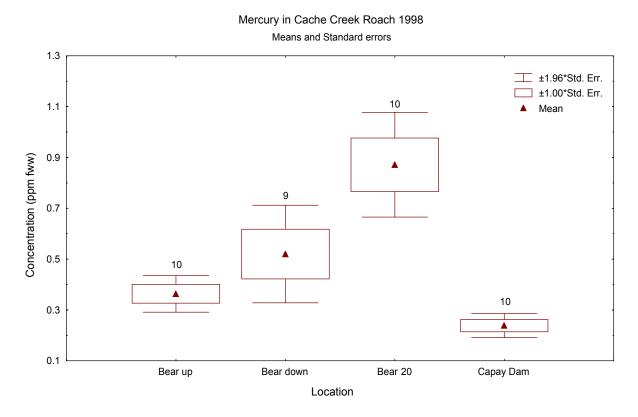


Figure 7.

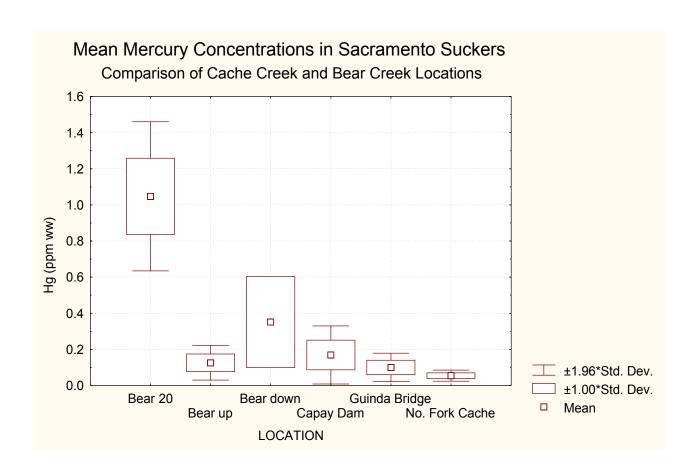


Figure 8.

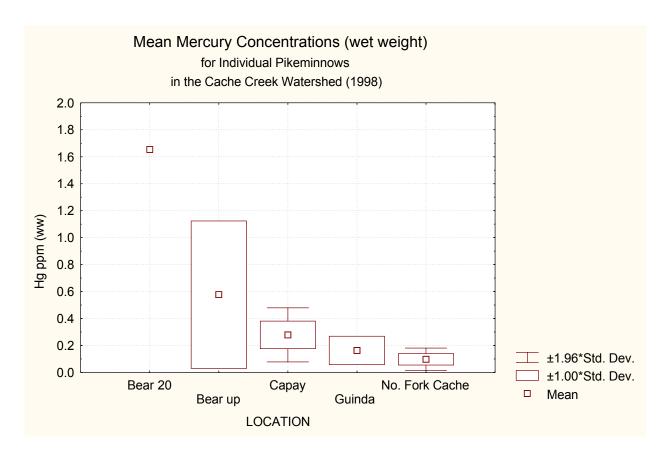


Figure 9

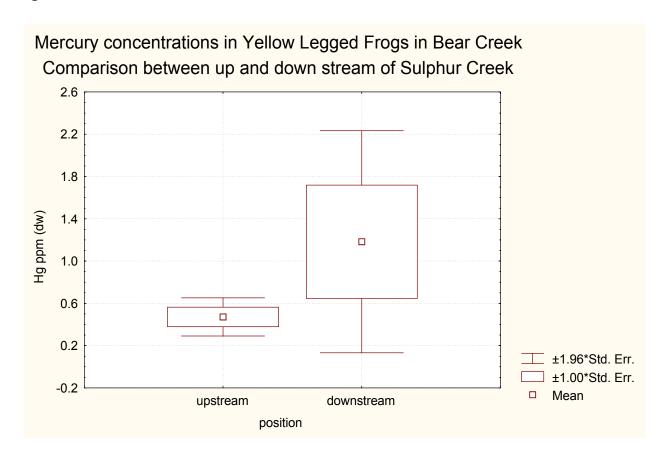


Figure 10.

Mercury Residues in Caddisfly Larvae collected in Bear and Cache Cr. 1997

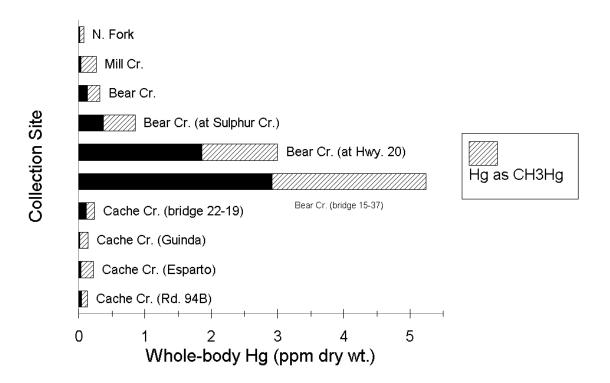


Figure 11.

Mercury Residues in Dragonfly Larvae collected in Bear and Cache Cr. 1997

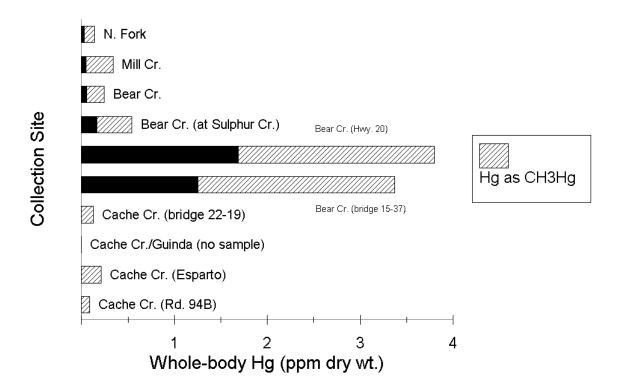


Figure 12

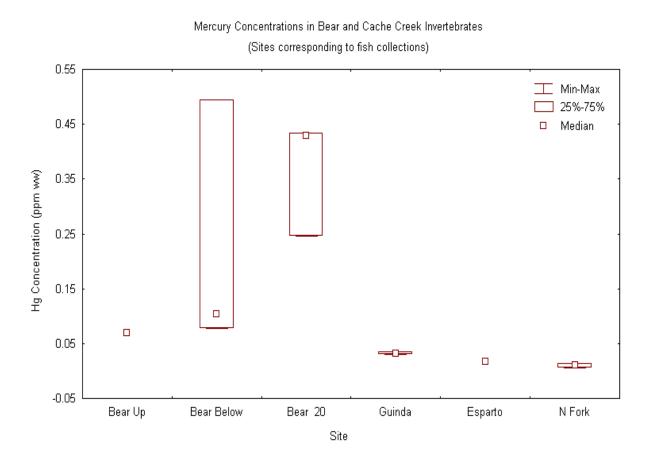


Figure 13.

Percent Methylmercury versus Whole-body Total Hg, Mixed Inverts

